

**Preliminary Amendment**

Applicant(s): Fred E. REGNIER et al.

Serial No.: 09/849,924

Filed: 4 May 2001

For: AFFINITY SELECTED SIGNATURE PEPTIDES FOR PROTEIN IDENTIFICATION AND QUANTIFICATION



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**Remarks**

The above amendments to the specification were made to correct obvious typographical and grammatical errors in the text. Amendments were also made to correct typographical errors in certain document citations. In each case, each citation provided sufficient information to determine the correct citation. Additionally, the correction at page 67, line 8, including –affinity ligands)– after "(endogenous" was necessitated due to a typographical error. Support for this correction may be found, for example, in the specification at page 23, lines 7-9.

No new matter was added as a result of the above amendments.

The Examiner is invited to contact Applicants' Representatives at the below-listed telephone number, if there are any questions regarding this Preliminary Amendment or if prosecution of this application may be assisted thereby.

**CERTIFICATE UNDER 37 C.F.R. 1.8:**

The undersigned hereby certifies that this paper is being deposited in the United States Postal Service, as first class mail, in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on this 11 day of January, 2002.

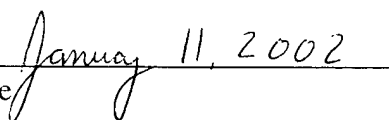
  
David L. Provence

Respectfully submitted for

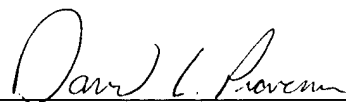
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APPENDIX A - SPECIFICATION AMENDMENTS  
INCLUDING NOTATIONS TO INDICATE CHANGES MADE

Serial No.: 09/849,924

Docket No.:290.0001 0101

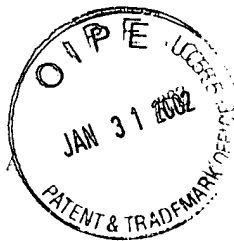
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Amendments to the following are indicated by underlining what has been added and bracketing what has been deleted. Additionally, all amendments have been indicated in bold.

**In the Specification**

The paragraph at page 3, lines 1-14, has been amended as follows:

Proteins in complex mixtures are generally detected by some type of fractionation or immunological assay technique. The advantages of immunological assay methods are their sensitivity, specificity for certain structural features of antigens, low cost, and simplicity of execution. Immunological assays are generally restricted to the determination of single protein analytes. This means it is necessary to conduct multiple assays when it is necessary to determine small numbers of proteins in a sample. Hormone-receptor association, enzyme-inhibitor binding, DNA-protein binding and lectin-glycoprotein association are other types of bioaffinity that have been exploited in protein identification, but are not as widely used as immunorecognition. Although not biospecific, immobilized metal affinity chromatography (IMAC) is yet another affinity method that recognizes a specific structural element of polypeptides (J. Porath et al., *Nature* 258: 598-599 ([1992] 1975)).



The paragraph at page 3, lines 15-29, has been amended as follows:

The fractionation approach to protein identification in mixtures is often more lengthy because analytes must be purified sufficiently to allow a detector to recognize specific features of the protein. Properties ranging from chemical reactivity to spectral characteristics and molecular mass have been exploited for detection. Higher degrees of purification are required to eliminate interfering substances as the detection mode becomes less specific. Since no single purification mode can resolve thousands of proteins, multidimensional fractionation procedures must be used with complex mixtures. Ideally, the various separation modes constituting the multidimensional method should be orthogonal in selectivity. The two-dimensional (2D) gel electrophoresis method of [O'Farrell] O'Farrell (*J. Biol. Chem.* 250:4007-4021 (1975)) is a good example. The first dimension exploits isoelectric focusing while the second is based on molecular size discrimination. At the limit, 6000 or more proteins can be resolved. 2D gel electrophoresis is now widely used in proteomics where it is the objective to identify thousands of proteins in complex biological extracts.

The paragraph at page 27, lines 9-15, has been amended as follows:

Tryptophan is present in most mammalian proteins at a level of <3%. This means that the average protein will yield only a few tryptophan containing peptides. Selective derivatization of tryptophan has been achieved with 2,4-dinitrophenylsulfenyl chloride at pH 5.0 (M. Wilcheck et al., Biochem. Biophys. Acta [178] 278:1-7 (1972)). Using an antibody directed against 2,4-dinitrophenol, an immunosorbant was prepared to select peptides with this label. The advantage of tryptophan selection is that the number of peptides will generally be small.



**Preliminary Amendment - Appendix A**

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The paragraph at page 31, lines 5-27, has been amended as follows:

After peptides of interest are detected using mass spectrometry, the protein from which a peptide originated is determined. In most instances this can be accomplished using a standard protocol that involves scanning either protein or DNA databases for amino acid sequences that would correspond to the proteolytic fragments generated experimentally, matching the mass of all possible fragments against the experimental data (F. Hsieh et al., Anal. Chem. 70:1847-1852 (1998); D. Reiber et **[all] al.**, Anal. Chem. 70:673-683 (1998)). When a DNA database is used as a reference database, open reading frames are translated and the resulting putative proteins are cleaved computationally to generate the reference fragments, using the same cleavage method that was used experimentally. Likewise, when a protein database is used, proteolytic cleavage is also performed computationally to generate the reference fragments. In addition, masses of the reference peptide fragments are adjusted as necessary to reflect derivatizations equivalent to those made to the experimental peptides, for example to include the exogenous affinity tag. The presence of signature peptides in the sample is detected by comparing the masses of the experimentally generated peptides with the masses of signature peptides derived from putative proteolytic cleavage of the set of reference proteins obtained from the database. Software and databases suited to this purpose are readily available either through commercial mass spectrometer software **[and] or** the Internet. Optionally, the peptide databases can be preselected or reduced in complexity by removing peptides that do not contain the amino acid(s) upon which affinity selection is based.



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The paragraph at page 61, lines 1-3, has been amended as follows:

2. Cysteine can be derivatized with an affinity tagged maleimide. Normal and deuterium labeled tags are mixed so that tagged species are easily identified in the MALDI spectrum as a doublet that is three mass units apart.

The paragraph at page 62, lines 1-2, has been amended as follows:

3. Cysteine can alternatively be derivatized with 2,4-dinitrobenzyl chloride. Conditions: pH 5, 1 hour, room temperature.



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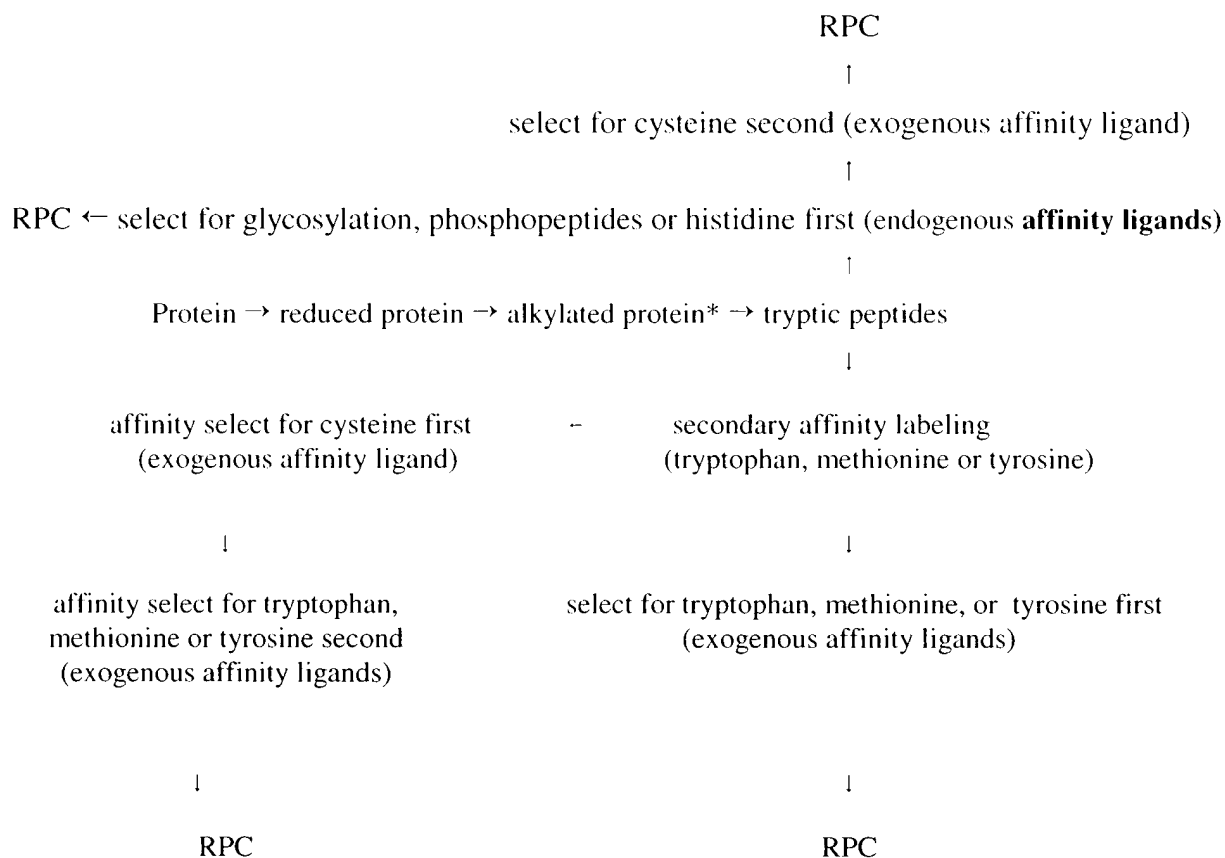
Filed: 4 May 2001

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The paragraph at page 67, lines 5-19, has been amended as follows:



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The paragraph at page 70, lines 10-19, has been amended as follows:

Data are presented that suggest proteins may indeed be quantified as their signature peptides by using isotopically labeled internal standards. Signature peptides generated by trypsin digestion have a primary amino group **[pat] at** their amino-terminus in all cases except those in which the peptide originated from the blocked amino-terminus of a protein. The specificity of trypsin cleavage dictates that the C-terminus of signature peptides will have either a lysine or arginine (except the C-terminal peptide from the protein) and that in rare cases there may also be a lysine or arginine adjacent to the C-terminus. Primary amino groups of peptides were acylated with *N*-hydroxysuccinimide.